REMARKS

Claims 1 to 3 and 5 will be pending in the application upon entry of the above amendments. Claims 1 to 3 and 5 have been amended. Support for the amendments to claims 1 to 3 and 5 exists in the specification as filed and in the knowledge attributable to one of ordinary skill in the art. Favorable reconsideration in light of above amendment and the remarks which follow is respectfully requested.

With regard to the amendments to claim 2, Applicants have specified that the drug amikacin is utilized to treat the infected cells specified in claim 2. Given the knowledge attributable to those of skill in the art and the bacteria specified in the application as filed, one of ordinary skill in the art would readily recognize that the step of treating the infected cells with a drug or drugs must result in cell death (see page 11, line 10, where the application as filed states that none of the cells in the vaccine are viable). Since the infected cells must be rendered non-viable, one of ordinary skill in the art would look to amikacin given the nature of the cell lines disclosed in the originally filed application. Accordingly, no new matter is believed to have been added to claim 2. As such, consideration of currently amended claim 2 is hereby respectfully requested.

I. 35 U.S.C. § 112, First Paragraph Rejection:

Claims 1 to 3 and 5 have been rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not adequately described in the specification in such a way as to enable one skilled in the art to make and/or use the invention.

With regard to this rejection the Examiner has maintained a number of issues, which include: (1) that the specification teaches treatment of only two bacteria, *M. tuberculosis* and *S. typhimurium*; (2) the specification provides no data resulting from the procedures disclosed therein relating to the treatment of the above bacteria in mice (as such the Examiner contends that there is no actual vaccine evidence); and (3) that due to the lack of evidence/data relating to other diseases the specification is non-

enabling for vaccines against these diseases as well.

With regard to the above rejection, the Examiner's attention is directed to the following. Applicants would like to submit that the approach of the present invention to develop vaccine is based on the fact that even though about one-third of the world population is infected with *M. tuberculosis*, only 5 to 10% develop active tuberculosis and thus 90 to 95% of the individuals develop effective and protective immunity against *M. tuberculosis*.

Thus, Applicants' approach to develop a vaccine utilizes the fact that *M. tuberculosis*, when present in a host macrophage, secretes unique antigens, which are or can be effective inducers of long lasting protective immunity in 90 to 95% of individuals. These individuals would then remain resistant against the pathogen throughout their life span. The failure of different vaccines tried to date is due in large part to the reason that *M. tuberculosis* when cultured *in vitro* in an artificial medium does not secrete antigens that induce an optimum level of protection. Therefore, any immunity that is generated by such a vaccine is short lived.

One of the advantages of the present invention in the development of a vaccine is that protective antigens are secreted by *M. tuberculosis* in a natural habitat inside the macrophages. It is these antigens which are utilized in the present invention. Hence, a vaccine produced in accordance with the present invention provides similar type of antigens that are being used by the immune system of resistant human beings in imparting protective immunity. Another important feature in a vaccine produced in accordance with the present invention is that antigens are used in their natural condition without subjecting the antigens to any chemical treatment in order to isolate the antigens from the macrophages.

Further, the use of syngeneic macrophages not only helps in providing the source of protective antigens but also would work as antigen presenting cell to generate tuberculosis reactive effector T-helper and cytotoxic cells.

Another problem in using a vaccine in human is the non-availability of adjuvant that can be used effectively and safely. The allo-macrophages used in vaccines in accordance with the present invention not only function as a unique vehicle for delivering antigens secreted by live mycobacterium to dendritic cells but also function as an important source of an adjuvant for eliciting the secretion of cytokines viz. IL-2, IL-12 and IFN-y from allo-reactive T cells. The excess secreted IL-2 cytokines is utilized by the mycobacterium reactive protective T cells. Therefore, there is no need for the use of any adjuvant. Alloreactive T cells produce chiefly IL-2, IFN-y and IL-12, the cytokines responsible for the generation of Thl-like immune response. Th1 cells are crucial for inducing protective immunity against *M. tuberculosis.*

The Applicants of the present application have actual data available for vaccines against two bacteria, *M. tuberculisis* and S. *typhimurium*, which have been successfully treated and both of them have been used as model systems. This invention successfully shows the hypothesis and gives the Applicants a reason to systematically extend this work to other bacteria. The actual vaccine data is available in the confidential laboratory data book of the inventors and will be submitted in the form of a Declaration by one of the named inventors within the Suspension of Action period.

The quantity and range of experimentation which is necessary to create evidence of actual protection from infection or protection from disease has been carried out in accordance with the methods set forth in the pending claims. The experimental data will be detailed in the aforementioned Declaration .

As would be apparent to one of ordinary skill in the art, the experiments conducted on *M. tuberculosis* and *S.typhimurium* is not limited in scope to these two bacteria alone, but can also be applied to systems having similar physiology such as *M. leprae*, leishmnia, other salmonella, trypanosoma, brucetla, listeria and HIV. The methodology established by the Applicants in the present invention has applicability to the above-mentioned systems.

The Applicants' wish to emphasize the following points in relation to generating favorable immune response using a vaccine generated in accordance with the present invention:

1) The allogeneic macrophages utilized by the present invention function as a unique system for delivering antigens secreted by live mycobacterium to dendritic cells and as an adjuvant for eliciting the secretion of cytokines (e.g., IL-2, IL-12, IFN-γ, etc.) from allo-reactive T cells. The excess secreted IL-2 cytokine was utilized by the mycobacterium reactive protective T cells. Therefore, there is no need for any adjuvant to be used. Alloreactive T cells produce chiefly IL-2, IFN-γ and IL-12, the cytokines responsible for the generation of Th1-like immune response. Th1 cells are crucial for inducing protective immunity against M. *tuberculosis.*

2) It is difficult to activate antigen reactive naive T cells unless the antigen is presented by dendritic cells. In the present invention, Applicants have used γ-irradiated mycobacterium infected macrophages. The γ-irradiated cells are known to undergo apoptosis. Apoptotic cells are engulfed by the dendritic cells that induce the activation of $CD4^+$ Th1 and $CD8^+$ cytotoxic T cells. Cytotoxic T cells are responsible for the killing of macrophages infected with mycobacterium. Lysis of targets is essential in the case of diseases like tuberculosis, typhoid, leprosy, leishmaniasis and AIDS, where the pathogen reside and multiplies within the macrophages. Lysis of these cells liberates the pathogen and gives an opportunity to activated macrophages to engulf the bacteria/pathogen and eliminate it. Dendritic cells express high level of B7-1 and secrete IL-12 and are only potent ARC that can activate naive T cells. Moreover, dendritic cells can

differentiate naive T cells to Th1 and CDS* cytotoxic T cells. TM and cytotoxic T cells are vital for induction of protective immunity against *M. tuberculosis*.

3) *M. tuberculosis* present in the host macrophages secretes unique antigens, which are the effective inducers of long lasting protective immunity. The outstanding feature in the process of the present invention is that the protective antigens of mycobacterium secreted inside the macrophages are being utilized that induces protective and long lasting immunity in 90 to 95% of individuals infected with mycobacterium tuberculosis.

4) Another advantage and unique feature of the present invention is that the dendrites on dendritic cells trap the foreign antigens and work as a reservoir. This antigen is slowly released from the dendrites, and is responsible for the maintenance of memory cells.

5) The AMTV vaccine works in a MHC-unrestricted manner, because it is based on allo-stimulation and engulfment of apoptotic cells by dendritic cells. It will work for all humans, irrespective of the genetic diversity.

6) Applicants have amikacin and isoniazid for killing *M. tuberculosis.* These are well-established drugs, widely known and utilized by those of ordinary skill in the art for such purposes and proven to have potent activity. For killing Salmonella, Applicants gamma irradiate the cells.

In view of the above and the aforementioned Declaration, one of ordinary skill in the art would recognize, upon reading and understanding the specification as originally filed, that the present invention does indeed permit one to produce a vaccine against tuberculosis and other intracellular infections/diseases.

This is because the application as filed discloses how to produce a vaccine against: (1) TB and other intracellular pathogens (Example 1), (2) TB (Example 2), and (3) salmonella, and as noted above one of ordinary skill in the art would readily recognize that the methodology of the present invention can be applied to other "systems". Accordingly, upon reading and understanding the application as filed, one of ordinary skill in the art would readily appreciate how to utilize the disclosed method to produce vaccines against the a wide variety of diseases as recited in claims 1 to 3 and 5.

As is well known, the Applicants are permitted to claim their invention as broadly as is supported by the specification and figures as filed. Accordingly, absent any specific evidence to the contrary, the Applicants believe that claims 1 to 3 and 5 are fully enabled by the application as originally filed. Accordingly, withdrawal of the 35 U.S.C. § 112, first paragraph rejections of claims 1 to 3 and 5 is respectfully requested.

II. 35 U.S.C. § 112, Second Paragraph Rejections:

Claims 1 and 5 have been rejected under 35 U.S.C. § 112, second paragraph, as indefinite. Specifically, the Examiner contends that the use of the term "cancer" in claims 1 and 5 render such claims indefinite.

Claims 1 and 5 have been amended to delete the term "cancer". Accordingly, it is respectfully submitted that the 35 U.S.C. § 112, second paragraph, rejection of claims 1 and 5 has been rendered moot. Thus, withdrawal thereof is respectfully requested.

III. Conclusion:

In view of the above, withdrawal of the above-mentioned rejections and allowance of claims 1 to 3 and 5 is respectfully requested.

Should the Examiner believe that a telephone interview would be helpful to expedite favorable prosecution, the Examiner is invited to contact Applicants' undersigned attorney at the telephone number listed below.

In the event any fees are due in connection with the filing of this document, the Commissioner is authorized to charge those fees to our Deposit Account No. 18-0988, Attorney Docket No. **KUMAP0105US**.

Respectfully submitted,
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